

Chapter 2

Senescence Phenotypes Induced by Ras in Primary Cells

Lena Lau and Gregory David

Abstract

Cellular senescence is defined as a state of stable cell-cycle arrest that is distinct from quiescence and terminal differentiation. Many stimuli can induce senescence, including telomere shortening and oncogene activation. The phenotypes elicited by pro-senescent signals can be heterogeneous depending on the stimulus and the cell type affected. To date, there is not a definitive marker that can ubiquitously and specifically mark all senescent cells. Therefore, several independent markers must be utilized to ascertain the senescent state of a cell or group of cells. Here, we describe common assays used to assess oncogenic Ras-induced senescence.

Key words Senescence, Oncogene, Ras, Primary fibroblasts, SASP, SA- β gal, SAHF

1 Introduction

Cellular senescence is a stable cell-cycle arrest that has been hypothesized to serve as a barrier to tumorigenesis. A strong piece of evidence that supports this hypothesis is that ectopic expression of activated oncogenes in primary cells induces senescence both in vitro and in vivo, thus preventing the propagation of potentially damaged cells [1–3]. To study the mechanisms and characteristics of oncogene-induced senescence, a commonly used in vitro system is the overexpression of an oncogenic form of Ras (H-Ras^{V12}) in human primary fibroblasts. Activation of oncogenic Ras induces the MAPK signaling pathway, leading to uncontrolled proliferation and hyper-replication of DNA [4]. This hyper-replication eventually leads to replication fork collapse, which is recognized as a sustained form of DNA damage and triggers cellular senescence.

Ras can be introduced into primary cells by retroviral infection. Constitutively active and inducible Ras constructs are readily available. Inducible Ras chimeric proteins consist of the activated oncogene fused to the ligand-binding domain of estrogen receptor (ER), whereby addition of 4-hydroxytamoxifen (4-OHT) stabilizes the fusion protein and thus induces Ras activity. We use the

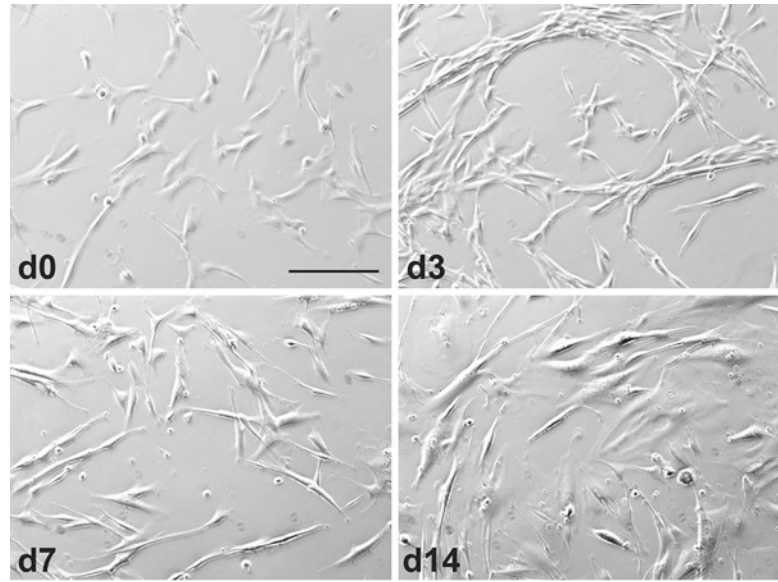


Fig. 1 Telomerase reverse transcriptase (TERT)-immortalized primary human lung embryonic fibroblasts (IMR90T) expressing an inducible form of H-Ras^{V12} (RasERT2) treated with 4-OHT for 0, 3, 7, and 14 days (d0, d3, d7, and d14, respectively). Cells proliferate rapidly and become spindly and refractile by day 3, start to senesce by day 7, and become fully senescent by day 14. Scale bar = 200 μ m

H-Ras^{V12}-ER^{T2} (RasERT2) construct, in which 100–125 nM 4-OHT is sufficient for potent oncogenic Ras activation (*see* Fig. 1). Since 4-OHT is diluted in ethanol (EtOH), control cells are treated in tandem with the same amount of EtOH. Upon oncogenic Ras activation, cells first undergo a hyper-replicative phase and become spindly and refractile when observed under a light microscope (*see* Fig. 1). Cells begin to senesce by day 6 and become fully senescent by day 14 [5, 6] (*see* Fig. 1). We find that 7 days of oncogenic Ras activation is sufficient to induce the senescence phenotypes described below, and therefore subsequent analyses are carried out at this time point.

Cellular senescence is characterized by a heterogeneous phenotype. Markers used to identify senescent cells are not exclusively present in senescent cells, and most senescent cells do not display all the markers of senescence. Therefore, multiple markers must be used in order to definitively confirm that senescence has been induced. In this chapter, we detail some of the most common markers and methods used to assay for oncogenic Ras-induced senescence. A brief description of each of each is given below.

Senescence-associated β -galactosidase (SA- β gal): SA- β gal positivity is a widely accepted marker of cellular senescence. SA- β gal is a senescence byproduct, as there is no evidence of any causative

effect of SA- β gal on senescence. Indeed, the gene encoding this protein is dispensable for senescence and other senescence-associated phenotypes [7]. The expression and activity of this enzyme is upregulated upon lysosomal stress and therefore is unlikely to mark senescent cells exclusively. However, it remains a very useful indicator of senescence when used concomitantly with other markers. SA- β gal activity is detected by incubating cells in acidic conditions with the synthetic substrate 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal), which, when hydrolyzed, causes the cells to appear blue [8].

Cell-cycle exit: The presence of nondividing cells can be detected by visualizing the lack of incorporation of 5-Bromo-2-Deoxyuridine (BrdU). BrdU is a synthetic thymidine analog that is only incorporated into cells actively synthesizing DNA. Therefore, nondividing cells will stain negative for BrdU. BrdU is most commonly detected in immunofluorescence experiments using anti-BrdU antibodies. This assay, along with the detection of increased cell-cycle exit markers (e.g., p16^{INK4A}, p21^{CIP1}) and decreased proliferation markers (e.g., Ki67), can be used to identify nonproliferating cells, a prerequisite for senescence.

Senescence-associated heterochromatin foci (SAHF): Senescent human cells have been shown to display distinct puncta in the nucleus that can be easily detected using 4',6-diamidino-2-phenylindole (DAPI) staining [9]. These nuclear foci represent domains of heterochromatin and are believed to help prevent the spreading of DNA damage throughout the chromatin fiber [10]. One caveat with this marker is that its use is restricted to human cells, as proliferating murine cells readily display nuclear foci reminiscent of SAHF. As with all other markers of senescence, not all senescent cells display SAHF, and therefore cells should not be automatically considered non-senescent due to its absence.

Senescence-associated secretory phenotype (SASP): Senescent cells remain metabolically active and secrete a wide array of growth factors, proteases, and cytokines collectively termed the senescence-associated secretory phenotype (SASP). The SASP is hypothesized to reinforce and maintain senescence, as well as communicate the senescent state to neighboring cells [11, 12]. The SASP is also hypothesized to recruit immune cells to the area, facilitating the clearance of damaged and potentially cancerous cells [13, 14]. Whether the SASP encompasses a similar set of cytokines regardless of the pro-senescence stimulus or the cell of origin remains to be fully elucidated. The SASP is most commonly detected using quantitative real-time PCR (qRT-PCR) or by enzyme-linked immunosorbent assays (ELISA). We choose to detect SASP using qRT-PCR. Genes commonly used to detect the presence of the SASP in multiple settings are listed in Table 1.

Table 1
Common SASP and cell-cycle exit genes used to detect senescence in human cells, along with their qRT-PCR primer sequences (see Note 17)

<i>Gene name</i>		<i>Primer sequence</i>
IL-1 α	F	GGT AGT AGC AAC CAA CGG GA
	R	TGG GTA TCT CAG GCA TCT CC
IL-6	F	CCA GGA GCC CAG CTA TGA AC
	R	CCC AGG GAG AAG GCA ACT G
IL-8	F	TTG GCA GCC TTC CTG ATT TC
	R	TCT TTA GCA CTC CTT GGC AAA AC
Gro- α	F	TTC ACC CCA AGA ACA TCC AA
	R	CTC CTA AGC ATG CTC AAA CAC
IL-1 β	F	GGC CCT AAA CAG ATG AAG TGC T
	R	TGC CGC CAT CCA GAG G
LMNB1	F	AAG CAG CTG GAG TGG TTG TT
	R	TTG GAT GCT CTT GGG GTT C
p16	F	CCC CTT GCC TGG AAA GAT AC
	R	AGC CCC TCC TCT TTC TTC CT
p21	F	CCT GTC ACT GTC TTG TAC CCT
	R	GCG TTT GGA GTG GTA GAA ATC T
Ki67	F	CTG GGT TAC CTG GTC TTA GTT C
	R	GAG GCT GTT CCT TGA TGA TTT

2 Materials

Prepare all reagents using double-distilled water (ddH₂O) unless otherwise indicated. Prepare and store all reagents at room temperature unless otherwise indicated. Note that some of the reagents are best prepared fresh before each use (these are indicated below).

2.1 SA- β gal Assay

1. Poly-L-lysine-coated coverslips

For Fixing Solution

2. 37 % formaldehyde solution, in H₂O.
3. 25 % glutaraldehyde solution, in H₂O. Store at 4 °C.
4. 2.0 % formaldehyde, 0.2 % glutaraldehyde in 1 \times PBS. For 6 mL of solution, add 320 μ L of 37 % formaldehyde and 48 μ L of 25 % glutaraldehyde into a 15 mL conical tube. Add 5.632 mL 1 \times PBS for a final volume of 6 mL.

5. Phosphate-buffered saline (PBS).

For SA- β gal Staining Solution

6. 5 \times citric buffer, pH 6.0: 126 mM sodium phosphate, dibasic (Na_2HPO_4), 36.7 mM citric acid (anhydrous, enzyme grade), and sodium hydroxide (NaOH) to pH (To make 250 mL of 5 \times citric buffer, dissolve 1.763 g citric acid and 4.472 g Na_2HPO_4 in 250 mL of ddH₂O. pH with NaOH dropwise up to 6.0.) (*see Note 1*). Filter solution through a 0.22 μM filter to sterilize. Store at 4 °C.
7. 20 mg/mL 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal) solution: Make a stock solution of 20 mg/mL in *N,N*-Dimethylformamide (DMF). For a 10 mL stock, add 200 mg X-gal powder to 10 mL of DMF. Store both X-gal powder and stock solution in the dark at -20 °C.
8. 0.5 M potassium ferricyanide. To make a 50 mL solution, add 8.232 g of potassium ferricyanide to 50 mL ddH₂O. Filter solution through a 0.22 μM filter. Store at 4 °C.
9. 0.25 M potassium ferrocyanide. To make a 50 mL solution, add 5.280 g of potassium hexacyanoferrate (II) trihydrate to 50 mL ddH₂O (*see Note 2*). Filter solution through a 0.22 μM filter. Store at 4 °C.
10. 5 M sodium chloride (NaCl).
11. 1 M magnesium chloride (MgCl_2).
12. 1 \times citric buffer, pH 6.0, 150 mM NaCl, 2 mM MgCl_2 , 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, and 1 mg/mL X-gal in ddH₂O. For a 6 mL solution, add 1.2 mL of 5 \times citric buffer, 180 μL of 5 M NaCl, 12 μL of 1 M MgCl_2 , 60 μL of 0.5 M potassium ferricyanide, 120 μL of 0.25 M potassium ferrocyanide, and 300 μL of 20 mg/mL X-gal solution to a 15 mL conical tube. Add 4.128 mL H₂O for a total volume of 6 mL.
13. Mounting medium with 4',6-diamidino-2-phenylindole (DAPI). Store at 4 °C.
14. Glass slides.
15. Nail polish.
16. Slide microscope with bright-field light and UV lamp and filter with excitation wavelength of 360 nm.

2.2 BrdU Incorporation

1. Poly-L-lysine-coated coverslips.
2. 30 mM 5-Bromo-2-Deoxyuridine (BrdU). To make a 10 mL solution, add 92.1 mg of BrdU to 10 mL of sterile ddH₂O. Aliquot into 1 mL tubes. Store aliquot that is currently being used away from light at 4 °C. Store the rest away from light at -20 °C.

3. 4% paraformaldehyde (PFA) solution in PBS. Store at 4 °C.
4. PBS.
5. 4 N hydrochloric acid (HCl). For a 6 mL solution, add 2 mL of concentrated HCl (12.1 N) to 4 mL of ddH₂O.
6. 1 M sodium tetraborate, pH 8.5. For a 100 mL solution, dissolve 2.012 g sodium tetraborate powder in 100 mL ddH₂O (*see Note 3*). pH down to 8.5 dropwise using concentrated HCl.
7. 0.1% Tween-20 with PBS (PBST). For a 1 L solution, add 1 mL Tween-20 to 1000 mL of PBS.
8. Permeabilization buffer: 0.5% Triton X-100 and 3% bovine serum albumin (BSA, heat shock treated) in PBST (*see Note 4*).
9. Primary antibody: anti-BrdU antibody. Store away from light at 4 °C.
10. Secondary antibody: goat anti-mouse IgG secondary antibody, Alexa Fluor488 conjugate (*see Note 5*). Store away from light at 4 °C.
11. Mounting media with DAPI. Store at 4 °C.
12. Glass slides.
13. Nail polish.
14. Slide microscope with UV lamp and filters with excitation wavelengths of 360 and 488 nm.

2.3 SAHF Formation

1. Poly-L-lysine-coated coverslips.
2. PBS.
3. Fixing solution (*see Subheading 2.1*).
4. Mounting medium with DAPI.
5. Glass slides.
6. Nail polish.
7. Slide microscope with UV lamp and filter with excitation wavelength of 360 nm.

2.4 Gene Expression via qRT-PCR (SASP, Cell-Cycle Exit Genes)

2.4.1 Total RNA Extraction

1. TRIzol.
2. Chloroform.
3. Isopropanol.
4. Ethanol, 70 %.
5. ddH₂O.

2.4.2 cDNA Synthesis Using Oligo dT and Reverse Transcriptase

1. DNase and DNase stop solution.
2. Oligo dT, 0.05 µg/mL.
3. Dithiothreitol (DTT), 0.1 M.

4. Deoxynucleotide mix (dNTPs), 10 mM.
5. RNase inhibitor.
6. Reverse transcriptase and buffer.
7. ddH₂O.

2.4.3 Quantitative Real-Time PCR (qRT-PCR)

1. Primers for genes of interest.
2. Maxima SYBRgreen Master Mix.
3. ddH₂O.
4. Real-time-compatible PCR plates.

3 Methods

3.1 SA- β gal Assay

1. Drive cells into senescence by activating oncogenic Ras. For inducible RasERT2, add 100 nM tamoxifen to media. Change media and add fresh tamoxifen every other day.
2. The day before harvest, plate 50,000 cells onto coverslips in a well of a 12 well plate in the presence of 4-OHT (*see* **Notes 6 and 7**).
3. On the day of harvest, prepare fixing solution: This should be made fresh right before use (*see* **Notes 8 and 9**).
4. Assay is performed on benchtop at the end of the day (*see* **Note 8**). Aspirate medium from the cells. Wash each well with 1 mL PBS. Aspirate. Wash each well a second time with 1 L PBS. Aspirate.
5. Incubate cells with 750 μ L of freshly made fixing solution for 10 min on a rocker at room temperature.
6. While cells are fixing, prepare SA- β gal staining solution.
7. Aspirate fixing solution. Wash each well with 1 mL PBS, aspirate. Repeat for a total of two washes.
8. Add 1 mL SA- β gal staining solution to each well.
9. Incubate cells in a humidified chamber at 37 °C overnight (*see* **Note 8**).
10. The next morning, aspirate staining solution. Wash each well once with 1 mL ddH₂O. Add 1 mL ddH₂O to each well to prevent cells from drying out.
11. Mount coverslips on slides with mounting medium containing DAPI.
12. Seal coverslips with nail polish.
13. Visualize cells under a slide microscope equipped with a UV lamp and bright-field light. DAPI-positive nuclei will be visible under UV fluorescence of 360 nm, while SA- β gal positivity will be visible under normal bright-field light. SA- β gal-positive cells will appear bright blue (*see* **Fig. 2**). To quantify, count 200

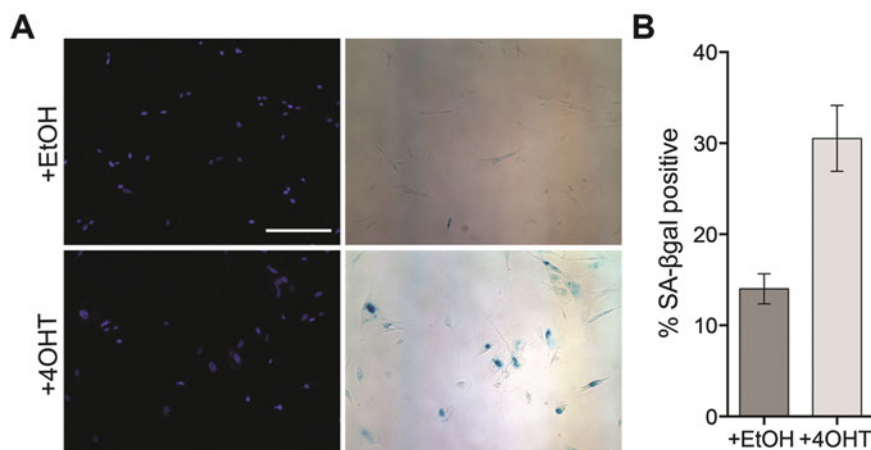


Fig. 2 SA-βgal positivity of IMR90T expressing RasERT2 treated with either EtOH or 4-OHT for 7 days. (a) Representative images of cells cultured in the presence of EtOH or 4-OHT fixed on day 7. *Left*: DAPI-positive nuclei under UV fluorescence. *Right*: SA-βgal staining of cells under bright-field light. Scale bar = 200 μm. (b) Quantification of SA-βgal positivity in cells treated as indicated. $n = 4$

DAPI-positive nuclei per coverslip. Switch to normal bright-field light and count SA-βgal-positive cells. Calculate percentage of SA-βgal-positive cells.

14. Store slides at 4 °C away from light.

3.2 BrdU Incorporation

1. Drive cells into senescence by activating oncogenic Ras. For inducible RasERT2, add 100 nM tamoxifen to media. Change media and add fresh tamoxifen every other day.
2. The day before harvest, plate 50,000 cells onto coverslips in a well of a 12 well plate (*see* **Notes 6** and **7**).
3. The next day, incubate cells with 30 μM BrdU for 2 h in normal culture conditions. In a well containing 2 mL of media, add 2 μL of 30 mM BrdU. Do not change media.
4. All steps hereafter are performed on the benchtop at room temperature. Aspirate media. Wash each well three times with 1 mL PBS.
5. Fix the cells with 500 μL 4% PFA per well. Rock plate for 10 min at room temperature.
6. Aspirate PFA. Wash each well once with PBS (*see* **Note 10**).
7. Incubate cells with 1 mL 4 N HCl for 10 min, rocking (*see* **Note 11**).
8. Aspirate. Neutralize with 1 mL 0.1 M sodium tetraborate. Rock for 7 min (*see* **Note 12**).
9. Aspirate. Wash each well with 1 mL PBS. Rock for 5 min.
10. Aspirate. Incubate cells in 1 mL of permeabilization buffer. Rock for 5 min.

11. Aspirate. Wash each well twice with PBS.
12. Aspirate. Wash each well once with PBST.
13. Dilute anti-BrdU antibody 1:100 in PBST. For 3 mL of primary antibody dilution, add 30 μ L anti-BrdU to 3 mL of PBST in a 15 mL conical tube.
14. Aspirate PBST from wells. Add 500 μ L of primary antibody dilution to each well. Rock for 1 h and 15 min. Cover plate with foil or keep plate in a dark container.
15. Aspirate. Wash each well three times with PBS.
16. Aspirate. Wash each well once with PBST.
17. Turn lights off (*see Note 13*). Dilute Alexa Fluor-conjugated anti-mouse antibody 1:500 in PBST. For 6 mL of secondary antibody dilution, add 12 μ L antibody to 6 mL of PBST in a 15 mL conical tube.
18. Aspirate PBST from wells. Add 1 mL secondary antibody dilution to each well. Rock for 1 h. Keep plate away from light.
19. Turn lights off (*see Note 13*). Aspirate. Wash each well three times with PBS.
20. Mount coverslips on slides with mounting medium containing DAPI.
21. Seal coverslips with nail polish.
22. Visualize cells under a slide microscope equipped with a UV lamp and filters with excitation wavelengths of 360 and 488 nm. DAPI-positive nuclei will be visible under the 360 nm filter. Switch to filter of excitation wavelength 488 nm to visualize BrdU positivity. Positive nuclei will be green (*see Fig. 3*). To

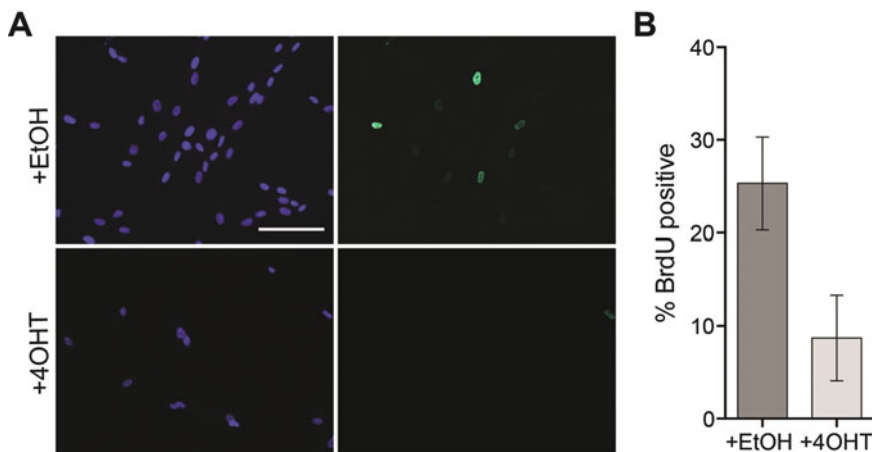


Fig. 3 BrdU incorporation of IMR90T expressing RasERT2 after treatment with either EtOH or 4-OHT for 7 days. **(a)** Representative images of cells cultured for 7 days in the presence of EtOH or 4-OHT after a 2 h incubation with BrdU. *Left*: DAPI-positive nuclei. *Right*: BrdU-positive nuclei, indicative of DNA synthesis. Scale bar = 100 μ m. **(b)** Quantification of BrdU incorporation in cells treated as indicated. $n=3$

quantify, count 200 DAPI-positive nuclei per coverslip. Count how many of those nuclei stain green. Calculate percentage of cells that are positive for BrdU (*see* **Note 14**).

23. Store slides at 4 °C away from light.

3.3 SAHF Formation

1. Drive cells into senescence by activating oncogenic Ras. For inducible RasERT2, add 100 nM tamoxifen to media. Change media and add fresh tamoxifen every other day.
2. On the day before harvest, plate 50,000 cells onto coverslips in a well of a 12 well plate (*see* **Notes 6** and **7**).
3. On the day of harvest, aspirate media from cells. Wash each well twice with 1 mL of PBS.
4. Incubate cells in 1 mL of fixing solution for 10 min, rocking (*see* **Note 15**).
5. Aspirate fixing solution. Wash each well twice with 1 mL of PBS.
6. Mount coverslips onto slides using mounting medium containing DAPI. Seal with nail polish.
7. Visualize cells under a slide microscope equipped with a UV lamp and filter with excitation wavelength of 360 nm. To quantify, count 200 cells per coverslip, and calculate percentage of cells that are positive for SAHF (*see* Fig. 4).
8. Store slides at 4 °C away from light.

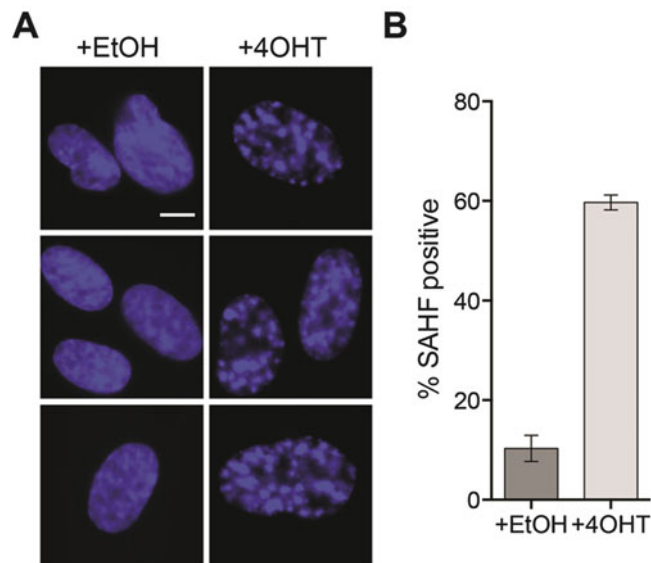


Fig. 4 Presence of SAHF in nuclei of IMR90T expressing RasERT2 treated with 4-OHT for 7 days. **(a)** Representative images of cells after 7 days of EtOH or 4-OHT treatment and stained with DAPI. Scale bar = 10 μ m. **(b)** Quantification of SAHF positivity in cells treated as indicated. $n=3$

3.4 Gene Expression via qRT-PCR (SASP, Cell-Cycle Exit Genes)

1. Drive cells into senescence by activating oncogenic Ras. For inducible RasERT2, add 100 nM tamoxifen to media. Change media and add fresh tamoxifen every other day.
2. Harvest cells into TRIzol.
3. Perform total RNA extraction.
4. Convert RNA into cDNA using reverse transcriptase.
5. Perform qRT-PCR for cell-cycle exit genes and SASP genes. Commonly used genes used to characterize senescent cells, along with their primer sequences, are listed in Table 1 (see Note 16).
6. Compare gene expression levels of cells treated with 4-OHT to control cells treated with EtOH (see Fig. 5).

4 Notes

1. It is critical for the pH of citric buffer to be 6.0. Physiological levels of lysosomal β gal activity will be detected at pH 4.0, the normal pH of lysosomes. At a suboptimal pH of 6.0, the assay will only detect cells with increased β gal levels, a characteristic of senescent cells.
2. A 0.5 M potassium ferrocyanide solution tends to crystallize at 4 °C. Making a more dilute concentration (0.25 M) will mitigate this problem. If crystals do form, warm the solution at 37 °C and vortex until crystals disappear before adding to SA- β gal staining solution.
3. Sodium tetraborate does not dissolve readily in water. To help it dissolve, stir the solution on a heated plate at 90 °C.
4. Permeabilization buffer should be made fresh before use. For 6 mL of buffer, dissolve 180 mg of BSA and add 6 μ L of Triton X-100 to 6 mL of PBST in a 15 mL conical tube.
5. Different colors of fluorescent secondary antibodies may be used, but we find that green fluorescence is brightest.
6. Some primary human cell lines (e.g., IMR90) have difficulty adhering to poly-L-lysine-coated coverslips. To assist with cell adhesion, incubate coverslips with 0.2 % gelatin in ddH₂O for at least 30 min at room temperature. Aspirate gelatin and let coverslips dry before cell plating.
7. Cells should always be plated in duplicate for experimental replicates.
8. SA- β gal staining should be performed in the evening. Staining will become visible by 3 h, but overnight incubation is required for complete staining. However, too long of an incubation will overstain the cells.

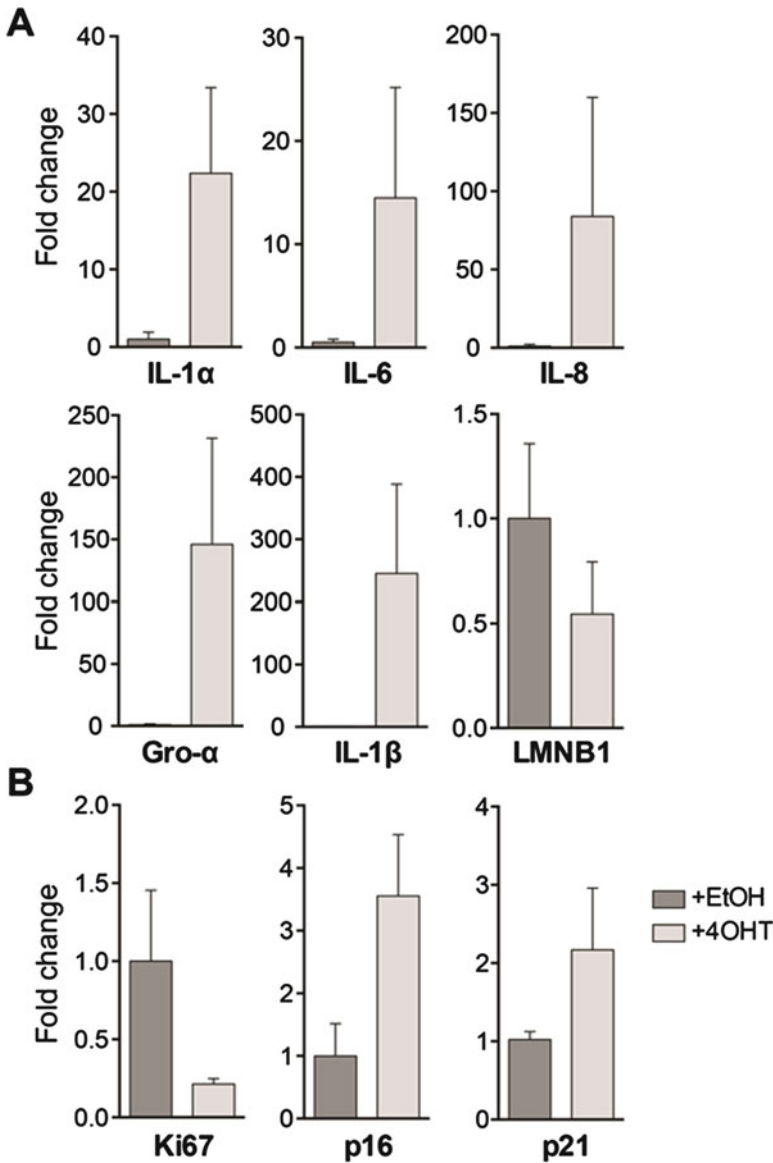


Fig. 5 mRNA expression levels of the indicated genes in IMR90T expressing RasERT2 treated with either EtOH or 4-OHT for 7 days. **(a)** mRNA expression levels of the indicated SASP factors (IL-1 α , IL-6, IL-8, Gro- α , and IL-1 β) and of lamin B1 (LMNB1), a component of the nuclear envelope. **(b)** mRNA expression levels of the indicated cell proliferation and cell-cycle exit markers. Values were normalized to tubulin. $n=4$ for SASP factors, $n=3$ for LMNB1, Ki67, p16, and p21

- 9. 4% PFA can also be used to fix cells, but fixing solution better preserves cell morphology.
- 10. After fixation and wash, cells can be stored for up to 2 weeks at 4 °C before performing immunofluorescence. Add 1 mL of

PBS to each well, seal plate with parafilm, and wrap in foil before storing at 4 °C.

11. During this incubation or during the neutralization step, prepare permeabilization buffer (*see* Subheading 2.2, **item 8**).
12. We find that neutralizing for 2 min causes DAPI to look brighter but BrdU to look dimmer. Neutralizing for 7 min results in a brighter BrdU stain, but DAPI becomes dimmer.
13. Overexposure to light will quench fluorescent molecules. Therefore, when using fluorescent secondary antibodies, minimize exposure to light.
14. For optimal fluorescence, analyze samples within 1 week.
15. We find that DAPI staining is brighter when cells are fixed with fixing solution compared to 4% PFA. Therefore, we recommend using fixing solution to fix cells for SAHF analysis.
16. SASP factors such as IL-1 α , IL-1 β , IL-6, IL-8, and Gro- α are upregulated in senescent cells. The nuclear protein lamin B1 (LMNB1) has been shown to be downregulated upon senescence (*see* ref. 15). Cell-cycle exit genes such as p16^{INK4A} and p21^{CIP1} are upregulated, while the replication marker Ki67 is downregulated in senescent cells.

References

1. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16^{INK4a}. *Cell* 88(5):593–602
2. Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM et al (2005) BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 436(7051):720–724
3. Collado M, Gil J, Efeyan A, Guerra C, Schuhmacher AJ, Barradas M et al (2005) Tumour biology: senescence in premalignant tumours. *Nature* 436(7051):642
4. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C et al (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* 444(7119):638–642
5. Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF et al (2009) Autophagy mediates the mitotic senescence transition. *Genes Dev* 23(7):798–803
6. Chen H, Ruiz PD, McKimpton WM, Novikov L, Kitsis RN, Gamble MJ (2015) MacroH2A1 and ATM play opposing roles in paracrine senescence and the senescence-associated secretory phenotype. *Mol Cell* 59(5):719–731
7. Lee BY, Han JA, Im JS, Morrone A, Johung K, Goodwin EC et al (2006) Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. *Aging Cell* 5(2):187–195
8. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C et al (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* 92(20):9363–9367
9. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA et al (2003) Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 113(6):703–716
10. Di Micco R, Sulli G, Dobrev M, Lontos M, Botrugno OA, Gargiulo G et al (2011) Interplay between oncogene-induced DNA damage response and heterochromatin in senescence and cancer. *Nat Cell Biol* 13(3):292–302
11. Kuilman T, Michaloglou C, Vredeveld LC, Douma S, van Doorn R, Desmet CJ et al (2008) Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network. *Cell* 133(6):1019–1031

12. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP et al (2013) A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol* 15(8):978–990
13. Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C et al (2008) Senescence of activated stellate cells limits liver fibrosis. *Cell* 134(4):657–667
14. Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE et al (2013) Non-cell-autonomous tumor suppression by p53. *Cell* 153(2):449–460
15. Freund A, Laberge RM, Demaria M, Campisi J (2012) Lamin B1 loss is a senescence-associated biomarker. *Mol Biol Cell* 23(11):2066–2075

Oncogene-Induced Senescence

Methods and Protocols

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